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(57) Abstract

Treatments have been developed for lupus patients using either anti-ID antibodies to dsDNA to block anti-dsDNA antibodies and/or kill the B cells producing the anti-dsDNA antibodies or ribosomal protein S1 peptides immunoreactive with anti-dsDNA antibodies. Examples demonstrate that (1) anti-dsDNA antibodies are cross-reactive with ribosomal protein S1, (2) anti-dsDNA antibodies suppress protein synthesis, presumably through inhibition of mRNA translation initiation, and (3) a normal human sera contains an anti-idiotypic antibody (anti-Id) to anti-dsDNA antibodies isolated from SLE patients which blocked the interactions between the anti-Id antibody fragment (Fab2) and various anti-dsDNA preparations.

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METHOD FOR TREATMENT OF SLE

Background of the Invention

The present invention includes methods and reagents for treatment of Systemic Lupus Erythematosus ("SLE") patients characterized double stranded (ds) DNA by administration of reagents reactive with doublestranded DNA antibodies to alleviate damage resulting from the antibodies.

Relationship of antibodies to dsDNA, the RNAproteins Ro/SSA, La/SSB, U_1RNP, and Sm, and <u>clinical disease expression.</u>

The laboratory directed by Morris Reichlin at the Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, has been engaged in the study of autoimmune responses to RNAprotein antigens in SLE patients for over 20 years. Researchers have reported the initial descriptions of the Ro/SSA (Clark, G.M., Reichlin, M. and Tomasi, T.B. *J. Immunol.*, 102:117-122 (1969)), La/SSB (Mattioli, M. and Reichlin, M. *Arthritis Rheum.*, 17:421-429 (1974)), and nRNP(U_1RNP) (Mattioli, M. and Reichlin, M. *J. Immunol.*, 107:1281-1290 (1971)) systems, while others described the Sm antigen (Tan, E.M. and Kunkel, H.G. *J. Immunol.*, 99:464-471 (1966)).

Over time, it has become apparent that certain profiles of anti-RNA protein antibodies are positively correlated with nephritis while other profiles are "negatively" correlated or "protected" from the development of serious renal disease. Thus, antibodies to nRNP(U_1RNP) alone were found to

have a low frequency of nephritis (Sharp, G.C., et al. *Am. J. Med.*, 52:148-159 (1972); Reichlin, M. and Mattioli, M. *N. Engl. J. Med.*, 286:908-911 (1972)) while patients with both anti-nRNP and anti-Sm (or anti-Sm alone) had a high frequency of nephritis (Reichlin, M. and Mattioli, M. *N. Engl. J. Med.*, 286:908-911 (1972); Maddison, P.J., et al. *J. Rheumatol.*, 5:407-411 (1978)). In patients with anti-Ro/SSA alone, a high frequency of nephritis was noted (Wasicek, C.A. and Reichlin, M. *J. Clin. Invest.*, 69:835-843 (1982); Hamilton, R.G., et al., *Arthritis Rheum.*, 31:496-505 (1988); Harley, J.B., et al. *Arthritis Rheum.*, 32(7):826-836 (1989)), while in those with both anti-Ro/SSA and anti-La/SSB, a low prevalence of nephritis was found. Studies of acid eluates from lupus nephritis kidneys have demonstrated enrichment of anti-Ro/SSA compared to serum levels (Maddison, P.J. and Reichlin, M. *Arthritis Rheum.*, 22:858-863 (1979)), supporting the participation of Ro/SSA-anti-Ro/SSA complexes in the development and/or the perpetuation of the nephritis. Elution studies of antibodies to the U_1RNP/Sm complex also showed enrichment, but the precise specificities of these complexes (anti-Sm or anti-nRNP) were not determined because of technical limitations (Koffler, et al. *J. Exp. Med.*, 134:294-312 (1971)). Serum levels of anti-Sm antibodies have been shown to fluctuate with disease activity (including nephritis) in some SLE patients (Barada, et al., *Arthritis Rheum.*, 24:1236-1244 (1981)). These data indicate a role for the Ro/SSA and Sm systems in the development of nephritis, but only 50% of patients with either anti-Ro/SSA alone or anti-nRNP and anti-Sm (or anti-Sm alone) develop nephritis.

        Much data support a major role for the DNA-anti-DNA system in the pathogenesis of lupus

nephritis. Clinical studies show that high serum
anti-DNA levels correlate positively with the
activity of nephritis, and that remissions are
associated with declining anti-DNA levels (Harley,
et al., *Arthritis Rheum.* (1989); Tan, et al. *J.
Clin Invest.*, 45:1732-1740 (1966); Schur, P.H. and
Sandson, J. *N. Engl. J. Med.*, 278:533-538 (1982)).
Anti-DNA has been shown to be enriched in serum
cryoglobulins (Winfield, et al., *J. Clin. Invest.*,
56:563-570 (1975)) and in acid eluates of lupus
nephritis kidneys (Maddison and Reichlin (1979);
Miniter, et al., *Arthritis Rheum.*, 22:959-968
(1979); Beaulieu, et al. *Arthritis Rheum.*, 22:565-
570 (1979)). In all these studies, the specificity
of these antibodies are to dsDNA (double stranded
or native DNA).

Antibodies to native or ds DNA play a
special role in the clinical diagnosis and
pathology of Systemic Lupus Erythematosus (SLE).
These autoantibodies are highly specific,
frequently correlate positively with disease
activity (especially nephritis), and remissions are
usually associated with declining anti-dsDNA levels
(Hahn and Tsao, Antibodies to DNA. -Tn Dubois,
Systemic Lupus Erythematosus. D- J. Wallace and B.
H. Hahn, editors. (Lea and Febiger, Philadelphia,
PA 1993) pp. 195-201; Harley, et al., *Arthritis
Rheum.* 32:826-836 (1989); Tan, et al., *J. Clin.
Invest.* 45:1732-1740 (1966); Schur and Sandson, *N.
Engl. T. Med.* 278:533-538 (1982)). Patients who
produce antibodies to the Ro/SSA and La/SSB
(Harley, et al. (1989); Wasicek and Reichlin, *J.
Clin. Invest.* 69:835-843 (1982); Hamilton, et al.,
Arthritis Rheum. 31:496-505 (1988); antigens as
well as those that only have precipitins to U_1RNP
(Sharp, Am. *J.* Med. 52:148-159 (1972); Reichlin and
Mattioli, N. *Engl. J. Med.* 86:908-911 (1972)) very

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infrequently have anti-dsDNA in their serum and
have a correspondingly low prevalence of nephritis.
The mechanisms of these negative relationships of
antibodies to Ro/SSA and La/SSB and U_1RNP with anti-
dsDNA are not understood.

Studies have been reported in the
literature describing differences in the ability of
murine monoclonal antibodies to dsDNA to induce
nephritis when hybridomas producing these
antibodies are placed in normal mice (Tsao, et al.
J. Clin. Invest., 85:530-540 (1990)). Others have
shown that murine monoclonal antibodies penetrate
cells, bind to nuclei, and induce glomerular
proliferation and proteinuria *in vivo* (Vlahakos, et
al., *J. Am. Soc. Nephrol.* 2:1345-1354 (1992)).
Most recently, others have reported direct *in vitro*
binding of murine monoclonal antibodies to
glomeruli which is DNA dependent (DiValerio, et
al., *Clin. Res.*, 42:139A (1994)).

Reichlin, et al., *The Immunologist* 3/3, 84-
88 (1995), characterizes anti-dsDNA antibodies as
cross-reactive with unfolded or denatured A and D
SnRNP polypeptides. Koren, et al., *J. Immunol.*
154:4857-4864 (1995), reported that murine and
human antibodies to native DNA that cross-react
with the A and D SnRNP polypeptides cause direct
injury of cultured kidney cells. However, many
questions remain about the mechanisms of lupus
nephritis and the role of antibodies to dsDNA.

It is therefore an object of the present
invention to provide methods and reagents for
neutralizing the pathogenicity of antibodies to
double stranded (ds) DNA.

It is another object of the present
invention to develop specific therapy based on
anti-idiotypes to anti-dsDNA.

Summary of the Invention

Treatments have been developed for lupus
patients using either anti-ID antibodies to dsDNA
to block anti-dsDNA antibodies and/or kill the B
cells producing the anti-dsDNA antibodies or
ribosomal protein S1 peptides immunoreactive with
anti-dsDNA antibodies. Examples demonstrate that
(1) anti-dsDNA antibodies are cross-reactive with
ribosomal protein S1, (2) anti-dsDNA antibodies
suppress protein synthesis, presumably through
inhibition of mRNA translation initiation, and (3)
a normal human sera contains an anti-idiotypic
antibody (anti-Id) to anti-dsDNA antibodies
isolated from SLE patients which blocked the
interactions between the anti-Id antibody fragment
(Fab$_2$) and various anti-dsDNA preparations.

Brief Description of the Drawings

Figure 1 is a graph of the inhibition of
antibody binding to the recombinant protein (G7-RP)
by DNA in ELISA. 5.0 ,µg/ml of G7-RP was used to
coat the ELISA plates. 33.C9 (●), 33.H11(O),
patient sera LG (▲) and JP (△), anti-dsDNA(AP) (■),
and anti-dsDNA(IK) (□) were preincubated with 0,
0.1, 1.0, 10, 100, or 1,000 µg/ml of calf thymus
dsDNA (-----) or Yeast tRNA (- - -) before being
added to the plates.

Figures 2a and 2b are the nucleotide and
deduced amino acid sequences of the cDNA insert in
G7. Asterisks show the stop codon. This sequence
has been added to the GenBank nucleic acid sequence
database, Los Alamos National Laboratory, NM, and
has been assigned accession number U27517.

Figures 3a and 3b are an alignment of the
central core regions of 5 ribosomal proteins (r-
proteins)

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S1. Asterisks show the 5 repeating regions (a, b,
c, d, and e, respectively). Spaces indicate
positions where gaps were introduced to optimize
alignment of the sequences. Dashes indicate
identity to the residues of HS1. Alignment of the
central core region of HS1 is residues 63-317.
HS1; human r-protein S1 presented in this study,
ES1; E. coli r-protein S1 (Ref. 26), RS1; Rhizobium
melilotii r-protein S1 (Ref. 28), PS1, Providencia
sp. r-protein S1 (Ref. 27), CS1; chloroplast r-
protein S1 (Ref. 29).

Figures 4A-D are graphs comparing the
affinity of 33.H11 and 33.C9 for G7-RP or DNA in
inhibition ELISA. 33.H11 [A (0.0031 μg/ml) and C
(1.0 μg/ml)] and 33.C9 [B (0.32 μg/ml) and D (0.56
μg/ml)] were preincubated with different
concentrations of calf thymus dsDNA (●), G7-RP (O),
Yeast tRNA (▲), or BSA (△), before adding the
antibodies to the plates coated with calf thymus
dsDNA (A and B) or G7-RP (C and D).

Detailed Description of the Invention

Therapeutic Applicants and Pharmaceutical
Compositions

Based on the results in the examples, one
can prepare anti-Id reagents (for anti-dsDNA) that
can be used to downregulate the production of anti-
dsDNA.

In one embodiment, reagents that are anti-
idiotypic antibodies to anti-dsDNA could be used to
down regulate or even curtail anti-dsDNA production
by SLE patients.

In a second embodiment, free peptide or a
conjugate of this peptide based on peptide sequence
of human ribosomal protein S1 can be used in
tolerance induction which could ablate anti-dsDNA.

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Peptide or Protein-based Compositions.

Attempts to influence anti-DNA production
in mouse lupus *in vivo* or in human lymphocytes *in
vitro*, are described by Borel, et al., *Science*,
182:76-77 (1973); Borel, et al., *J. Clin. Invest.*,
61:276-286 (1978); Borel, Y. and Borel, H., *J.
Clin. Invest.*, 82:1901-1907 (1988). As described
by Borel, et al., oligonucleotides or nucleosides
are attached to isologous (same species) IgG and
this is allegedly effective in (1) inhibiting the
development of an immune response to DNA in murine
lupus and decreasing disease severity, and
(2) inhibiting human cells from producing anti-DNA
in vitro. The "DNA" used by Borel is single
stranded or denatured which is not optimal since
the most important response in SLE is to native or
double stranded DNA. Borel's work provides an
appropriate "carrier" for the toleragen, isologous
gamma globulin.

As described herein, peptide(s) that are
immunoreactive with dsDNA and are derived from the
human ribosomal protein S1 can be used to induce
tolerance in a patient. Antibodies to dsDNA are
the disease specific pathogenic autoantibodies of
the greatest interest. There are two major
possibilities: (1) inject free peptide, or (2)
inject peptide-coupled to human IgG, for example,
coupled using glutaraldehyde or carbodiimide.
These two approaches should both induce T cell
tolerance. They may also be effective in inducing
B cell tolerance. Both approaches are attractive
since there is little chance of "boosting" the
anti-dsDNA response. Should the latter occur, it
can be treated by standard immunosuppressive drugs,
alone or in combination with anti-La/SSB or anti-
U_1RNP, as described below.

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Behavior of the peptide or peptide
conjugate is first studied in an appropriate animal
model in order to determine efficacy and optimal
dosages.   There are several that could be used, but
the most attractive is the Palmerston North Mouse.
It has been shown that these mice, which all
produce anti-dsDNA and develop nephritis, also
develop anti-U_1RNP and Sm responses in almost all
the animals with a dominant immune response against
the A protein of U_1RNP measured in Western blot, as
reported by Handwerger, et al., *Clin. Res.* 42:315A
(1994).   These mice have no detectable antibodies
in the first three months of life but rapidly
develop them after six months of age and experience
a fulminant glomerulonephritis associated with
anti-dsDNA antibodies.   Dosage would range from 3
to 300 micrograms per mouse given weekly in the
first experiments.

The same result obtained by administering
peptide or a peptide conjugate can be achieved by
coupling recombinant or isolated human ribosomal
protein S1 to human IgG.

Although described herein with reference to
the whole protein, it is preferable to use peptides
of between a few amino acids up to about 100 amino
acids, more preferably less than forty amino acids,
still more preferably less than ten to twenty amino
acids.   These peptides can be easily ascertained by
immobilizing the anti-dsDNA antibodies from a
patient(s) and screening for binding of the
peptides.   Peptides can be prepared using standard
techniques for amino acid synthesis or
recombinantly, by engineering the cDNA encoding the
protein, described in Figures 2a and 2b.

**Anti-Id Antibodies That Are Immunoeactive
With Anti-dsDNA Antibodies.**

As demonstrated by Example 3, normal human
sera contains anti-Id antibodies immunoreactive

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with anti-dsDNA antibodies present in many SLE
patients. Antibodies for use in treating patients
can be obtained using standard techniques to
harvest antibodies from normal people, or, more
preferably, antibody producing cells are isolated
by binding of cells expressing antibody using a
method as described in Example 3 for isolation of
antibody. The antibody producing cells are then
transformed with Eppstein-Barr virus (EBV),
amplified in culture, the gene encoding the
variable region of the anti-Id antibodies cloned,
inserted into an appropriate vector, and expressed
in bacteria or another appropriate expression
system, using known techniques. Preliminary
studies have yielded several clones.

        In either case, antibody is administered to
a patient in a dosage which decreases the amount of
anti-dsDNA antibody. This is readily determined
since SLE patients are routinely assayed for blood
levels of anti-dsDNA. In most cases patients are
expected to respond as they do to standard
immunosuppressive therapy, by decreasing production
of anti-dsDNA antibodies. In some cases, the
antibodies will result in killing of the antibody
producing cells in the patient. Treatments will be
repeated as required.

        An alternative approach is to screen
recombinant libraries of Ig variable ("V") regions
made from cDNA's reverse transcribed from mRNA
extracted from peripheral blood lymphocytes from
patients who produce anti-anti-dsDNA antibodies. A
number of such libraries can be constructed and
then screened for clones reactive with Fab anti-
dsDNA but not normal Fab. These can then be used
to produce any desired amount of anti-idiotype to
anti-dsDNA. Alternatively, murine recombinant
monoclonal anti-idiotypic antibodies directed

against relevant idiotope(s) on anti-dsDNA can be produced.

This can be accomplished by the use of Pharmacia's (Pharmacia LKB Biotechnology, Sweden) "Recombinant Phage Antibody System" (RPAS), which generates a single-chain Fv fragment (ScFv) that incorporates the complete antigen-binding domain of the antibody. In the RPAS, antibody variable heavy and light chain genes are separately amplified from the hybridoma mRNA and cloned into an expression vector. The heavy and light chain domains are co-expressed on the same polypeptide chain after joining with a short linker DNA which codes for a flexible peptide. This assembly generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody. Compared to the intact monoclonal antibody, the recombinant ScFv includes a considerably lower number of epitopes, and thereby presents a much weaker immunogenic stimulus when injected into humans. The murine ScFv molecules can be "humanized"to further reduce the immunogenic stimulus presented.

Methods for "humanizing" antibodies, or generating less immunogenic fragments of non-human antibodies, are well known. A humanized antibody is one in which only the antigen-recognized sites, or complementarily-determining hypervariable regions (CDRs) are of non-human origin, whereas all framework regions (FR) of variable domains are products of human genes.

These "humanized" antibodies present a lesser xenograft rejection stimulus when introduced to a human recipient.

To accomplish humanization of a selected mouse monoclonal antibody, the CDR grafting method described by Daugherty, et al., *Nucl. Acids Res.*,

19:2471-2476, 1991, incorporated herein by
reference, can be used. Briefly, the variable
region DNA of a selected animal recombinant anti-
idiotypic ScFv is sequenced by the method of
Clackson, T., et al., *Nature*, 352:624-688, 1991,
incorporated herein by reference. Using this
sequence, animal CDRs are distinguished from animal
framework regions (FR) based on locations of the
CDRs in known sequences of animal variable genes.
Kabat, H.A., et al.1, *Sequences of Proteins of
Immunological Interest, 4th Ed.* (U.S. Dept. health
and Human Services, Bethesda, MD, 1987). Once the
animal CDRs and FR are identified, the CDRs are
grafted onto human heavy chain variable region
framework by the use of synthetic oligonucleotides
and polymerase chain reaction (PCR) recombination.
Codons for the animal heavy chain CRDs, as well as
the available human heavy chain variable region
framework, are built in four (each 100 bases long)
oligonucleotides. Using PCR, a grafted DNA
sequence of 400 bases is formed that encodes for
the recombinant animal CDR/human heavy chain FR
protection.

The present invention will be further
understood by reference to the following examples.

**Example 1:   Anti-dsDNA antibodies are cross-
                reactive with human ribosomal protein
                S1.**

It has been reported that anti-dsDNA
antibodies cross-react with a number of proteins
(5-12). However, no one has reported using human
anti-dsDNA antibodies as probes to clone cDNAs
which encode proteins recognized by anti-dsDNA
antibodies. A cDNA clone that encodes human
ribosomal protein S1 which is recognized by anti-
dsDNA antibodies from SLE patients has been
isolated using this method.

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Materials and Methods

Abbreviations: SLE, systemic lupus
erythematosus; Anti-dsDNA(AP) and anti-dsDNA(IK),
affinity-purified anti-dsDNA antibodies from
patient sera AP and IK; GST, glutathione S-
transferase; Kd, dissociation constant; TBS, tris-
buffered saline; IFA, indirect immunofluorescence;
G7-FP, GST-fusion protein expressed by G7; G7-RP,
recombinant protein expressed by G7; r-proteins,
ribosomal proteins; ES1, E.coli ribosomal protein
S1; HS1, human ribosomal protein S1

Sera

Affinity-purified anti-dsDNA antibodies
were eluted from DNA cellulose column (Sigma
Chemical Company, St. Louis,MO) as described by
Reichlin, et al., *J. Clin. Invest.* 93:443 (1994).
Ten SLE patient sera containing anti-dsDNA
antibodies, two samples of affinity-purified anti-
dsDNA antibodies from patient sera AP and IK [anti-
dsDNA(AP) and anti-dsDNA(IK), respectively], and 2
human IgG monoclonal anti-dsDNA antibodies (33.C9
and 33.H11) (Winkler, et al. *Clin. Exp. Immunol.*
85:379 (1991)) were used for characterizing cDNA
clones. The isotypes and light chains of 33.C9 and
33.H11 were IgG2 (kappa) and IgG1 (lambda),
respectively. Both of these monoclonal anti-dsDNA
antibodies were derived from one SLE patient serum.
Anti-dsDNA antibodies were detected by the
Crithidia assay (Aarden, et al., *Ann. NY Acad. Sci.*
254:505 (1975)).

Screening of cDNA libraries

A phage lambda gt11 cDNA library
constructed from mRNA of human liver (Clontech
Laboratories Inc., Palo Alto, CA) was screened as
described by Young and Davis, *Proc. Natl. Acad.
Sci. USA* 80:1194 (1983) with a SLE patient (LG)
serum which contained a high titer of anti-dsDNA

antibody. Serum LG also contained anti-U1RNP and
anti-Ku antibodies. A positive plaque was
sequentially subcloned until all progency plaques
were recognized by the serum.

5   ·   Preparation of glutathione S-transferase
                        fusion protein and recombinant protein,
                        Western blot

         EcoRI-digested cDNA insert isolated from
the recombinant phage was ligated into EcoRI-
10 digested pGEX-1λT expression vector (Pharmacia LKB
Biotechnology Inc., Uppsala, Sweden). The
glutathione S-transferase (GST) fusion protein was
expressed in *E. coli* and purified by using
glutathione sepharose 4B as described by Smith, et
15 al., *Gene (Amst.)* 67:31 (1988). The GST carrier
protein was removed by thrombin proteolysis to
isolate the recombinant protein.
Western blot was performed as described by Itoh, et
al., *Clin. Exp. Immunol.* 81:45 (1990). In Western
20 blot, alkaline phosphatase-conjugated goat-anti-
human IgG (Sigma Chemical Company, St. Louis, MO)
was used as the second antibody. Patient sera were
diluted to 1:100 for use in Western blot.
                   Treatment of sera with DNAse I
25   To treat the sera with DNAse I, sera were
incubated with 10 μg/ml of DNAse I (Sigma Chemical
Company, St. Louis, MO) for 1 H at 37° before
incubating with nitrocellulose (NC) membranes in
Western blot.
30   Inhibition Western blot
         Inhibition Western blot reactivity of the
sera against the GST-fusion protein was
accomplished by preincubating with appropriate
dilutions of the sera for 3 h at room temperature
35 with 0, 5, 10, 50, or 100 μg/ml of calf thymus
dsDNA or Yeast tRNA (Sigma Chemical Company, St.

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Louis, MO) before incubating the sera with NC
membranes bound GST-fusion protein.

Inhibition ELISA

Purified recombinant protein (5.0 μg/ml in
0.05 M carbonate bicarbonate buffer, pH 9.6) was
coated on a microtiter plate well (Costar,
Cambridge, MA) by incubating overnight at 4°C. For
coating the plates with DNA, 0.5 mg/ml of protamine
sulfate was precoated by incubating for 3 h at room
temperature. After washing twice with 0.05% Tween
20 in PBS, 20 μg/ml of calf thymus dsDNA (Sigma
Chemical Company, St. Louis, MO) was coated by
incubating overnight at 4°C. Appropriate dilutions
of the sera were preincubated with different
concentrations of the recombinant protein, BSA,
calf thymus dsDNA, or Yeast tRNA (Sigma Chemical
Company, St. Louis, MO) for 3 h at room temperature
before applying the sera to the plates. After
blocking the plates with 0.1% BSA in PBS (4°C,
overnight), preincubated sera were added and
incubated (4°C, overnight). After washing 5 times
with 0.5% Tween 20 in PBS, alkaline phosphatase-
conjugated goat anti-serum IgG (Sigma Chemical
Company, St. Louis, MO) was added and incubated
(4°C, overnight). After washing 5 times with 0.05%
Tween 20 in PBS, p-nitrophenyl phosphate substrate
solution was added and the OD at 405nm as measured.
% inhibition was defined as follows; % inhibition
=

$$\frac{\text{OD without inhibitor } - \text{ OD with inhibitor}}{\text{OD without inhibitor}} \times 100$$

Calculation of dissociation constant of antibodies

The dissociation constant $(Kd)^3$ were
calculated by Scatchard analysis as described by
Friquet et al., J. Immunol. Methods. 77:305 (1985).

<u>Preparation of MOLT4 cell extract</u>

MOLT4 cells were collected, washed twice
with 0.02M Tris-buffered saline (TBS), and were
resuspended in 0.01M Tris-HCl, 0.015M NaCl, 0.0%
Nonidet P-40, pH 7.2. After centrifugation at
10,00g for 15 min, the supernatant was used as
MOLT4 cell extract.

Affinity purification of antibodies bound
<u>the GST-fusion protein</u>

After the purified GST-fusion protein was
electrophoresed and transferred to NC membranes,
membranes were blocked with 5% skim milk solution
in TBS. Membranes were then incubated with a
patient serum LG for 2 h at room temperature.
After washing 3 times with 0.05% Tween 20 in TBS,
membrane-bound antibodies were eluted by 0.5M
glycine-HCl, pH 2.9, neutralized by adding Tris
base, and then concentrated using Centricon 30
concentrator (Amicon Division, W.R. Grace, Denvers,
MA). These purified antibodies were used as probes
for Western blot using MOLT4 cell extract.

<u>DNA sequencing</u>

A cDNA insert isolated from a recombinant
phage (EcoRI fragments) was digested with several
restriction enzymes (Bgl II, EcoRV, Pst I, and
Sau3A I; Promega Corp., Madison, WI) and the
resulting DNA fragments were ligated into the
polylinker regions of M13mp18 replicative form DNA.
Nucleotide sequences were determined by using the
dideoxychain termination method (Sanger, et al.
Proc. Natl. Acad. Sci. USA 74:5463 (1977)) with T7
DNA polymerase (Dale, et al. *Plasmid* 13:31 (1985))
(U.S. Biochemical Corp., Cleveland, OH).
Nucleotide and amino acid sequence were analyzed
using the Sequence Analysis Software Package of the
Genetics Computer Group, University of Wisconsin

(Devereux, et al., *Nucleic Acids Res.* 12:387
(1984)) on a VAX 8250 computer.

Inhibition immunofluorescence

Appropriate dilutions of 33.C9 and 33.H11
were preincubated with 0, 1.0, 10, 100, or
1,000 µg/ml of the recombinant protein, BSA, calf
thymus dsDNA, or Yeast tRNA overnight at 4°C before
applying the antibodies for indirect
immunofluorescence (IFA)[3] using Hep 2 cells. IFA
using Hep 2 cells (INOVA Diagnostics, Inc., San
Diego, CA) was performed as described by Gonzalez,
et al., N.*Eng. J. Med.* 274:1333 (1966). FITC-
labelled goat anti-human IgG (Sigma, Chemical
Company, St. Louis, MO) was used at the second
antibody.

Results

Isolation and characterization of a cDNA
encoding a protein bound by anti-dsDNA
antibody

Using a SLE patient (LG) anti-dsDNA serum
as a probe, 1 x 10^{6} clones of the human liver cDNA
library in lambda gt11 phage were screend and one
positive clone (termed G7) obtained. This clone
was recognized by anti-dsDNA antibodies [anti-dsDNA
antibody-positive patient sera LG and AC, anti-
dsDNA (AP), and anti-dsDNA(1K) anti-bodies] when
the β-galactosidase fusion protein was induced by
isopropyl thiogalactoside. On the other hand,
normal control, anti-Ku, and anti-U1RNP antibody-
positive sera did not react with this fusion
protein. EcoRI-digestion of the recombinant phage
DNA demonstrated a 1.3-kb cDNA insert in G7.

To express the GST-fusion protein, the cDNA
insert was then ligated into pGEX-1λT expression
vector. A Western blot of the GST-fusion protein
expressed by G7(G7-FP) was carried out. G7-FP (66
kDa) was electrophoresed (10% polyacrylamide gel)

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and transferred to nitrocellulose membranes. NC
membranes were incubated with following sera; Anti-
dsDNA patient sera LG, BK, JP, WC, JH, RP, CC, JC,
BJ, and AC, anti-dsDNA(AP), anti-dsDNA(IK), 33.C9,
33.H11, normal control serum, and anti-U1RNP/Sm
antibody serum IA.

As shown on the Western blot, all the 10 patient
sera containing anti-dsDNA antibodies (diluted to
1:100), both of the affinity-purified anti-dsDNAs
(6.6 μg/ml), and both of the IgG human monoclonal
anti-dsDNA antibodies (27 μg/ml of 33.C9 and 1.8
μg/ml of 33.H11) recognized the 66 kDa GST-fusion
protein expressed by G7 (G7-FP)[3] (28 kDa of 66 kDa
is the GST portion and 38kDa is expected to be the
recombinant part encased by G7). None of them
reacted with GST carrier protein which was
expressed in *E. coli* transformed with non-insert
pGEX-1λT vector on Western blot. Inhibition of
antibody binding to the GST-fusion protein or the
recombinant protein by DNA was demonstrated by
Western blot. G7-FP (66 kDa) was electrophoresed
(10% polyacrylamide gel) and transferred to NC
membranes. 33.C9 (0.27 μg/ml) was preincubated
with 0, 5, 10, 50, or 100 μg/ml of calf thymus
dsDNA or Yeast tRNA before incubating with NC
membranes. As demonstrated by the Western blot,
33.C9 recognized G7-FP even if 33.C9 is diluted to
0.27 μg/ml. As there is a possibility that anti-
dsDNA antibodies bind to DNA in sera and this DNA
which made complexes with anti-dsDNA antibodies
binds to G7-FP, anti-dsDNA antibodies [33.C9 (27
μg/ml) and serum LG (diluted to 1:100)] were
treated with DNAse I. Treatment by DNAse I did not
decrease the reactivity of these antibodies against
G7-FP on Western blot. This result shows that
anti-dsDNA antibodies react directly with G7-FP.

To establish the specificity of antibodies
which recognize the 66 kDa G7-FP in Western blot as
anti-dsDNA antibodies, 33.C9 (0.27 μg/ml) was
preincubated with different concentrations of calf
5   thymus dsDNA or Yeast tRNA before incubating with
the NC membrane bound G7-FP. The reactivity of
33.C9 against G7-FP was inhibited by 5 μg/ml of DNA
but not by RNA.

G7-GP was proteolysed with thrombin to
10   isolate the recombinant protein (G7-RP).
Polyacrylamide/NaDodSO$_4$ gel electrophoresis (SDS-
PAGE) and Western blot analysis of Gy-RP solution
showed that only one recombinant protein band (42
kDa) was seen and was recognized by anti-dsDNA
15   antibody-positive patient serum LG, anti-dsDNA
(AP), anti-dsDNA (IK), 33.C9, and 33.H11 but was
not recognized by normal control, anti-U1RNP, nor
anti-Ku antibody-positive sera. Thus, one can
conclude that this recombinant protein solution
20   contains only G7-RP and has antigenicity.

The inhibition of antibody binding to G7-RP
by DNA was also examined in ELISA (Figure 1). Two
anti-dsDNA antibody-positive patient sera (LG and
JP diluted to 1:10^4), affinity-purified anti-dsDNA
25   antibodies [anti-dsDNA(AP) and anti-dsDNA(IK), both
of which were 0.33 μg/ml, and 33.H11 (0.0071 μg/ml)
were pre-incubated with different concentrations of
calf thymus dsDNA or Yeast tRNA before adding these
antibodies to the plates coated with G7-RP.
30   Reactivity of all these anti-dsDNA antibodies was
inhibited almost completely (% inhibition greater
than 90%) by DNA but not by RNA.

These results suggest that antibodies which
recognizes G7-FP or G7-RP are anti-dsDNA
35   antibodies.

Reactivities of the affinity-purified
antibodies eluted from G7-FP with MOLT4
cell extract.

G7-FP-transferred NC membrane was incubated
with serum LG. The antibodies that bound G7-FP
from this NC membrane were eluted. This affinity-
purified antibody at concentration of 5 μg/ml was
positive for anti-dsDNA antibody by the Crithidia
assay and recognized G7-FP on Western blot.

The affinity-purified antibody eluted from
G7-FP was used as a probe in Western blot to
identify the responsible protein which the cDNA
insert in G7 encodes. Reactivity of the affinity-
purified antibody eluted from G7-FP with MOLT4 cell
extract was determined by SDS-PAGE (5.5%
polyacrylamide gel) transferred to NC membranes.
The NC membranes were incubated with normal control
serum, affinity-purified antibody eluted from G7-
FP, patient sera LG, AC, and JP, anti-dsDNA(AP),
anti-dsDNA(IK), 33.C9, anti-U1RNP patient serum YN,
and anti-Ku patient serum HK. A common protein
band (104 kDa) was recognized by all the anti-dsDNA
antibodies and the affinity-purified antibody
eluted from G7-FP. The affinity-purified antibody
(from serum LG) at a concentration of 5 μg/ml
eluted from G7-FP reacted only with this 104kDa
protein.

On the other hand, normal control, anti-
U1RNP, and anti-Ku antibody-positive sera did not
react with this protein. Also in Western blot with
12.5% polyacrylamide gel using MOLT4 cell extract,
the affinity-purified antibody eluted from G7-FP
recognized only this 104kDa protein and this
protein band was commonly reactive with anti-dsDNA
antibodies (not shown). Thus, it appears that this
104 kDa protein at least in MOLT4 cell extract is

the responsible protein of which G7 encodes a
portion.

            Sequence analysis of the cDNA insert in G7
            The nucleotide sequence of the cDNA insert
in G7.was determined. Its primary nucleotide and
deduced amino acid sequences (GenBank no. U27517)
are shown in Figures 2a and 2b. The cDNA insert
proved to be 1,314 nucleotides in length. The TAA
stop codon is located at positions 1057-1059. The
predicted molecular weight for the encoded
polypeptides (352 amino acids) is 38.0 kDa.
However, this cDNA insert in G7 seems to be a
partial length cDNA because the molecular weight of
the encoded polypeptide is smaller than the
estimated full length size (104 kDa) of the
reactive protein in MOLT4 cell extract. Thus, this
cDNA does not seem to contain the initiation codon.
            A search for similarities between the
nucleotide sequence of the cDNA in G7 (GenBank no.
U27517) and other sequences through the NCBI using
he BLAST network service showed a significant match
(99% identity) with a sequence encoding human
ribosomal protein (r-protein) S1 homologue mRNA
reported by Eklund at al., Gene 155:231 (1995).
However, there are 3 nucleotide and 1 amino acid
differences between G7 and their cDNA sequence (GTC
(positions 130-132) in G7 vs GTA (positions 292-
294) in their cDNA, AGT (positions 133-135, encodes
Ser at residue 45) in Gy vs GCT (positions 295-297,
encodes Ala at residue 99) in their cDNA].
Moreover, 2 nucleotides (C at positions 1355 and
1366) and 162 nucleotides (positions 1-162) in
their cDNA are deleted in G7. A search was made
for some similarities between the predicted amino
acid sequence and other protein sequences in the
SWISSPROT database using the algorithm as described

by Gish, et al., *Nature Genetics* 3:266 (1993);
Altschul, et al., *J. Mol. Biol.* 215:403 (1990).

High degree of homology between the central
core region (residues 63-317) of the
predicted amino acid of this protein and
those of several r-proteins S1.

Identify and similarity with r-proteins S1
are the following; 39% identity and 65% similarity
with E. coli r-protein S1 (ES1) (26), 40% identify
and 64% similarity with Providencia sp. r-protein
s1 (Schnier, et al., *Mol. Gen. Genet.* 200:476
(1985)), 38% identity and 63% similarity with
Rhizobium melilotti r-protein S1 (Schnier, et al.,
Nucleic Acids Res. 16:3075 (1988)), and 50%
identity and 71% similarity with chloroplast r-
protein S1 (Franzetti, et al., *J. Biol. Chem.*
267:19075 (1992)). Moreover, 5 repeating regions
[EGTV (residue 158-161 and 243-246), DFGAFV (166-
171 and 251-256), GLVHVS (178-183 and 264-269),
GDKV (200-203 and 286-289), and RISLS (216-220 and
302-306)] were observed in the protein sequence.
These repeating residues have a high degree of
homology among other r-proteins S1 (Figures 3a and
3b).

Inhibition immunofluorescence

Inhibition of indirect immunofluorescence
was measured using Hep 2 cells. Staining of Hep 2
cells by 33.H11 or 33.C9 without preincubation,
after preincubation with G7-RP [100 μg/ml for
33.H11 or 1,000 μg/ml for 33.C9], or after
preincubation with calf thymus dsDNA [1,000 μg/ml
for 33.H11 or 1.0 μg/ml for 33.C9]. 33.H11 (0.18
μg/ml) without inhibitor added showed cytoplasmic
and nucleolar staining pattern while 33.C9 (1.7
μg/ml) showed homogenous nuclear staining pattern
in IFA. After preincubating with from 1.0 to 1,000
μg/ml of BSA or RNA as negative controls, these
staining patterns were not changed or diminished.

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The staining pattern of 33.H11 was inhibited by 100
and 1,000 μg/ml of G7-RP and by 1,000 μg/ml of calf
thymus dsDNA while that of 33.C9 was inhibited by
from 1.0 to 1,000 μg/ml of calf thymus dsDNA but
not by any concentration of G7-RP.

Comparison of the affinity of 33.h11 and
33.C9 against G7-RP or DNA by inhibition
ELISA

The inhibition of 33.H11 and 33.C9 binding
to G7-RP was compared by DNA or reciprocally
binding to DNA by G7-RP in inhibition ELISA
(Figures 4A-D). 33.H11 was diluted to 0.0031 μg/ml
and 1.0 μg/ml while 33.C9 was diluted to 0.32 μg/ml
and 0.56 μg/ml for ELISA using calf thymus dsDNA
and G7-RP as antigens, respectively. These
antibody concentrations were used as they gave 75%
of the maximum OD for the ELISA coated with calf
thymus dsDNA or G7-RP when saturating amounts of
antibody were used. These antibodies were
preincubated with from 0 to 1,000 μg/ml of G7-RP,
calf thymus dsDNA, bSA, or Yeast tRNA. The binding
of 1.0 μg/ml of 33.H11 to Gy-RP was not inhibited
by DNA (Figure 4C) while the binding of 33.C9 was
inhibited completely by DNA (Figure 4D). On the
other hand, in an ELISA with DNA-coated plates, the
inhibition of 33.C9 by G7-RP was far less effective
(Figure 4B) compared with that of 33.H11 (Figure
4A), as about 100 times more G7-RP was required for
inhibition. These data suggest that 33.H11 binds
G7-RP more strongly than does 33.C9 while 33.C9
binds DNA more strongly than does 33.H11.

Kd value of 33.C9 and 33.H11 for G7-RP or
DNA

From the data of the inhibition ELISA
coated with G7-RP (described as the open circles in
Figure 4C and 4D) and calf thymus dsDNA (described
as the closed circles in Figure 4A and 4B), the Kd

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value of each monoclonal anti-dsDNA antibody was
calculated for G7-RP and calf thymus dsDNA as the
ligands, respectively.

Before the calculation of Kd, average
molecular weight of the monomeric unit of calf
thymus dsDNA were determined because the size of
calf thymus dsDNA is heterogeneous. If
%inhibition/100 on the inhibition ELISA is defined
as v, K_D (dissociation constant) can be rewritten
from the Scatchard equation (Friguet, et al. 1985)
as follows;

$$K_D = a_0 - i_0 v) \ (1/v - 1)$$

where a_0 and i_0 are the total concentration of the
inhibitor (mol/l) and the total antibody
concentration (mol/l), respectively. Therefore, if
% inhibition at two different concentrations of the
inhibitor added and i_0 are known, a_0 (mol/l) and
molecular weight of the inhibitor can be determined
even if K_D is unknown. As a result, the average
molecular weight of the monomeric unit of calf
thymus dsDNA was 1.0×10^6.

K_d values of 33.c9 and 33.H11 for G7-RP or
DNA were calculated by Scatchard plots. 42,000 was
used as the molecular weight of G7-RP. As shown in
Table I, the K_d value of 33.H11 for G7-RP was lower
than that for DNA. On the other hand, K_d value of
33.C9 for DNA was two magnitudes lower than that
for G7-RP. As affinities are inversely
proportioned to the Kd value, these data suggest
that 33.H11 has a higher affinity for G7-RP than
for DNA while 33.C9 has a much higher affinity for
DNA than for G7-RP. These differences of
affinities correlate with the different staining
patterns of IFA exhibited by the 2 monoclonal anti-
dsDNA antibodies. In that view, 33.h11 binds the
cytoplasm plus nucleolus where one would expect the

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r-protein S1 to be localized and 33.C9 binds the
nucleus where DNA is localized.

Table I.   Kd (dissociation constant) (mol/l) of
              the two human IgG monoclonal anti-
              dsDNA antibodies for calf thymus dsDNA
              or G7-RP.

	ligand	
antibodies	calf thymus dsDNA	G7-RP
33.H11	1.0×10^{-7}	6.5×10^{-8}
33.C9	3.0×10^{-9}	3.9×10^{-7}

Discussion

        In summary, a lambda gt11 cDNA library
constructed from mRNA of human liver was screened
by using a SLE patient serum with anti-dsDNA
antibody and a clone G7 which has a 1.3-kb cDNA
insert isolated. Not only all of the 10 anti-dsDNA
patient sera but also affinity-purified anti-dsDNA
and human IgG monoclonal anti-dsDNA antibodies
recognized the protein expressed by G7. The
affinity-purified antibody eluted from this protein
was positive for anti-dsDNA antibody activity by
the Crithidia assay. Moreover, antibody binding to
this protein was inhibited completely by DNA but
not by RNA. From those observations, it was
concluded that anti-dsDNA antibodies cross-react
with the protein expressed by G7.

        A significant match (99% identify) between
the nucleotide sequence of the cDNA in G& and a
cDNA reported by Eklund et al. as encoding human r-
protein S1 homologue mRNA. It appears that anti-
dsDNA antibodies directly bind to the protein
expressed by G7 because the reactivity of anti-
dsDNA antibodies against the protein was not
influenced by DNAase I treatment and the binding of
anti-dsDNA antibodies to the protein was inhibited
completely by DNA. The predicted amino acid

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sequence presented in this study had homology with
some r-proteins S1 including ES1. ES1 is well
characterized at the functional and structural
level (Subramanian, *Prog. Nucleic Acids Res. Mol.
Biol.* 28:101 (1983)) while there are few reports
about mammalian r-proteins S1. ES1 is the largest
protein of the ribosome and has the same length as
the ribosome. This protein is associated with the
30S ribosomal subunit in prokaryotes via its N-
terminal globular domain and is known to stimulate
translation by facilitating mRNA binding to the 30S
ribosomal subunit. The central and C-terminal
region contain repeating homologous sequences which
are known to play a key role in the binding of
structural elements of r-protein S1 to mRNA. Five
repeating regions (EGTV, DFGAFV, GLVHVS, GDKV and
RISLS) which repeat twice in the central core
region of the protein were observed (Figures 3a and
3b). This apparent gene duplication which encodes
this repeat region is absent only in the
chloroplast r-protein S1.

Cytoplasmic and nucleolar staining patterns
in IFA suggests that the antigenic target is the
ribosome. This is the IFA pattern of human
monoclonal anti-dsDNA antibody 33.H11 which is
inhibited by both 100 μg/ml of G7-RP and higher
concentrations (1,000 μg/ml) of DNA. These results
show that G7 encodes a ribosomal protein which is
recognized by anti-dsDNA antibodies. From these
observations, it is concluded that G7 encodes a
part of human r-protein S1 (HS1).

It has been reported that anti-dsDNA
antibodies have high frequencies of basic amino
acids carrying positive charges in the heavy chain
complementarity determining regions and that
arginine is the most versatile amino acid for
binding with negative-charged DNA (31). However,

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there are no high scoring negative charged
segments, which could be an epitope for anti-dsDNA
antibody, in the primary sequence of HS1. These
observations might suggest that cross-reactions
between anti-dsDNA antibody and Hs1 are not
dependent on charge interaction in the primary
sequence alone but rather that the cross-reactive
epitope depends on conformational apposition of
negative charges in the tertiary structure of HS1.
However, it is likely that HS1 mimics DNA because
anti-dsDNA antibodies cross-react with HS1. It is
also appealing to believe that proteins which
"mimic" the structure of DNA could play a role as
immunogen.

          Both 33.H11 and 33.C9 are IgG monoclonal
anti-dsDNA antibodies (Winkler, et al., *Clin. Exp.
Immunol.* 85:379 (1991)) and strongly recognized the
protein expressed by G7. However, in IFA, 33.H11
did not show a homogeneous nuclear pattern and the
homogeneous nuclear staining patterns of 33.C9 was
not inhibited by G7-RP but was inhibited by as
little as 1.0 μg/ml of DNA. Also in ELISA, much
lower concentrations of DNA were able to inhibit
the binding of 33.C9 to G7-RP compared with that of
33.H11 while much higher concentrations of G7-RP
were needed to inhibit the binding of 33.C9 to DNA
compared with that of 33.H11. Although the binding
of 33.H11 (1.0 μg/ml) to G7-RP did not seem to be
inhibited even by 1,000 g/ml of DNA (Figure 4C),
that of 33.H11 (0.0071 g/ml) to G7-RP were
inhibited completely by DNA (Figure 1). Therefore,
it is likely that this concentration (1.0 g/ml) of
33.H11 in Figure 4C is too high to be inhibited by
DNA. Analysis of K_d value showed that 33.H11 has a
higher affinity for HS1 than for DNA while 33.C9
has a higher affinity has a higher affinity for DNA
than for HS1. In most anti-dsDNA-positive SLE

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patient sera, anti-dsDNA antibodies behave like
33.C9 which has a higher affinity for DNA than for
HS1 but indeed recognizes HS1. Anti-dsDNA
antibodies of this type likely predominate because
5   such sera rarely show a cytoplasmic and nucleolar
staining pattern but rather a classical nuclear
pattern but rather a classical nuclear pattern in
IFA as does 33.C9.

Yanase, et al. *Lab. Invest.* 71:52 (1994),
10   have reported that anti-dsDNA antibodies penetrate
living cell membranes and bind to cytoplasmic
proteins before binding to the nucleus. From these
standpoint, anti-dsDNA antibodies like 33.H11
recognize HS1 strongly and are trapped in the
15   cytoplasm preventing their entry into the nucleus.
In studies with living PK15 cells, 33.H11
penetrates the plasma membrane and indeed localizes
in the cytoplasm.

IT is assumed that most of the amino acid
20   sequences which G7 encodes are mRNA-binding sites
on HS1 because five repeating regions (residues
158-306) which repeat twice in HS1 and are supposed
to be a feature of the mRNA binding site are
observed. Therefore, if anti-dsDNA antibodies like
25   33.H11 bind to mRNA-binding portions on HS1, these
anti-dsDNA antibodies might block the binding of
mRNA to 40S (eukaryotes) ribosomal subunit, that
is, the initiation reaction of translation.

Example 2:   Suppression of Protein Synthesis by
30   **   anti-dsDNA antibodies cross-reactive**
**   with Ribosomal Protein S1.**

Four systemic lupus erythematosus (SLE)
patient sera containing anti-dsDNA Antibodies, 3
affinity-purified anti-dsDNA IgG, and a human
35   monoclonal anti-dsDNA Ab (33.H11) immunoprecipitate
18S ribosomal RNA from DNase-treated ^{32}P-labeled
MOLT4 cell extract. This 18S RNA precipitation was

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inhibited completely by preincubating 33.H11 with
calf thymus dsDNA or the recombinant human
ribosomal protein S1, which was described in
Example 1 as cross-reactive with anti-dsDNA

5   antibodies. 33.H11 did not immunoprecipitate 18S
RNA when deproteinized labeled cell extract was
used as the antigen. Whole IgG from 3 SLE sera
with anti-dsDNA Antibodies, 33.H11, and 3 affinity-
purified anti-dsDNA IgG inhibited in vitro

10   translation of globin mRNA (% inhibition was 36-
50%). This translation inhibition by anti-dsDNA
Antibodies was enhanced (67-79%) when the
reticulocyte lysate was treated with DNase.
Suppression of protein synthesis is thereby

15   indicated as a pathogenic mechanism of anti-dsDNA
Antibodies. The r-protein S1 in *E. coli* is well
known to be associated with the 30S ribosomal
subunit via its N-terminal globular domain. This
protein has mRNA-binding sites and plays a key role

20   in transaction initiation by binding to mRNA in *E.
coli*. In this study, anti-dsDNA Antibodies were
shown to immunoprecipitate 18S ribosomal RNA and
suppress *in vitro* translation of mRNA. These date
are the first to demonstrate inhibition of *in vitro*

25   protein synthesis by anti-dsDNA Antibodies.
Materials and Methods
        Recombinant r-protein S1
        Recombinant r-protein S1 (42 kDa) expressed
from G7 clone (G&-RP) was prepared as described in

30   Example 1.
        RNA immunoprecipitation (RNA-IP)
        A procedure based on that of Matter, *et al.
Arthritis Rheum.* 25:1278 (1982) was used. MOLT4
cells (2 x 10^6 cells) were labeled with 100 μCi of

35   ^{32}P-phosphate (ICN Pharmaceuticals Inc., Costa Mesa,
CA) in RPMI 1640 medium (phosphate-free) for 16 h.
Cells were washed with Tris-buffered saline (TBS),

lysed in 0.01 M tris-HCl, pH 7.2, 0.145 M NaCl,
0.5% Nonidet P-40 (NP-40) for 20 min, and
centrifuged at 10,000 g for 10 min. After
centrifugation, 100 μl of the supernatant was

5 incubated with 10 units of RQ1 DNase (RNase-free)
(Promega Corp., Madison, WI) for 30 min at 37°C and
was used as the labeled cell extract. Two mg of
Protein A Sepharose 4B (Pharmacia LKB Biotechnology
Inc., Uppsala, Sweden) preswollen in 500 μl of NET-

10 2 (0.01 M tris, 0.15 M NaCl, pH 7.5, 0.05% NP-40)
was incubated with an appropriate amount of
antibodies (10 μl for a serum) for 15 h at 4°C,
and then washed 5 times with NET-2. The antibody-
bound beads were incubated for 2 h at 4°C with the

15 labeled cell extract. After 5 washes with NET-2,
the beads were incubated with 1 unit of RQ1 DNase
for 30 min at 37°C. Precipitated RNAs were
extracted by phenol extraction and ethanol
precipitation and analyzed on polyacrylamide non-

20 denaturing gel electrophoresis (2.5% acrylamide,
0.04 M Tris-acetate, pH 7.8, 0.02 M NaOAc, 0.002 M
EDTA) (Loening, *Biochem. J.* 102:251 (1967))
followed by audioradiography.

In experiments designed to test whether

25 proteins are necessary for antibodies to
precipitate RNAs, labeled cell extract was
deproteinized by phenol extraction and ethanol
precipitation and then resuspended in 100 μl of
NET-2 before RNA-IP was performed as described

30 above.

Inhibition of immunoprecipitating ability
of 33.H11 by dsDNA or G7-RP

A quantity (0.1 μg) of the human IgG
monoclonal anti-dsDNA Ab 33.H11 (Winker, et al.

35 1991) was preincubated in 500 μl of NET-2 with 100
μg/ml of calf thymus dsDNA (or Yeast tRNA as a
negative control) or 100 μg/ml of G7-RP (or BSA as

a negative control) before RNA-IP was performed as described above. For RNA-IP by 33.H11 which was preincubated with calf thymus dsDNA or Yeast tRNA, labeled cell extract and washed beds were not

5   treated with RQ1 DNase.

<u>Anti-dsDNA sera and purification of IgG</u>

Anti-dsDNA antibodies were detected by the *Crithidia* assay or ELISA. For the affinity-purification of anti-dsDNA IgG, SLE patient sera

10   containing anti-dsDNA antibodies were applied to DNA cellulose columns (Sigma Chemical Company, St. Louis, MO) as described above and the eluate was incubated with 10 mg of Protein A Sepharose 4B in TBS for 15 h at 4°C. After washing 5 times with

15   TBS, IgG was eluted from the Ab-bound beads with 0.2 M glycine-HC1, pH 3.0 and concentrated after neutralization.

For purifying whole IgG from patient sera or for 33.H11 IgG, 50 μl of patient sera or 1 ml of

20   the supernatant from the hybridoma cell culture for 33.H11 were incubated with 10 mg of Protein A Sepharose 4B and IgG was eluted as described above. Five SLE patient sera containing anti-dsDNA antibodies which are positive with the *Crithidia*

25   assay [LG(anti-U1RNP) +), AP(anti-U1RNP +), CC (anti-Ro/SSA +), IK(anti-U1RNP +), JP(anti-dsDNA alone)], three samples of affinity-purified anti-dsDNA IgG from patient sera LG, Ap, and CC [anti-dsDNA(LG), anti-dsDNA(AP), and anti-dsDNA(CC),

30   respectively], and 33.H11 IgG were used as anti-dsDNA-positive samples. As negative controls, two normal human sera (DB and CW), SLE patient FF serum containing only antibodies to U1RNP and Sm (anti-U1RNP/Sm), and SLE patient NK serum containing only

35   antibodies to Ro/SSA and La/SSB (anti-Ro/La) were used. Three SLE patient sera (JC, AR, and MH) were used as anti-ribosomal protein P antibodies (anti-

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P) positive sera. Antibodies to UlRNP, Sm, Ro/SSA,
La/SSB, or ribosomal protein P were detected by
double immunodiffusion using bovine spleen or calf
thymus extract (Clark, et al., *J. Immunol.* 102:117
(1969)·; Mattioli and Reichlin, *Arthritis Rheum.*
17:421 (1974)). Human Cohn fraction II (Sigma) was
used for normal human IgG.

Inhibition of *in vitro* translation

A procedure based on Targoff, *et al., J.
Clin. Invest.* 84:162 (1989), was used, modified as
follows. A standard translation reaction using a
rabbit reticulocyte lysate system kit (Boehringer
Mannheim Corp., Indianapolis, IN) with rabbit
globin mRNA was set up, with [^{3}H]leucine (ICN) as
the labeled amino acid. Then μl of the rabbit
reticulocyte lysate was preincubated with 1 unit of
RQ1 DNase (RNase-free) (Promega) (or water alone as
negative control) for 30 min at 37°C. Purified IgG
was dialyzed against water and concentrated.
Lysate with or without DNase treatment was
preincubated with IgG solution (or water alone as
negative control) which contains 1 unit of RNase
inhibitor (Sigma) for 2 h at 4°C. After adding all
the reaction components and 5 μl of rabbit globin
mRNA (Life Technologies Inc., Gaithersburg, MD),
the lysate was incubated for 1 h at 30°C to start
translation (completing a 32.5- μl reaction
volume). After incubating with 3.25 μl of 1 mg/ml
RNase A for 15 min at 30°C, 5 μl of the reaction
was spotted on glass fiber filters. After washes
with 5% TCA and ethanol, cpm of the filters were
determined by liquid scintillation counting.
Translation reaction was determined as the average
of duplicate translation runs (cpm). Fidelity of
the replicates was assessed by calculating the
Pearson correlation coefficient. Percent
inhibition was defined as follows:

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$$\% \text{ inhibition} = \frac{\text{cpm after no IgG added} - \text{cpm after IgG added}}{\text{cpm after no IgG added}}$$

Results

    RNA immunoprecipitation by anti-dsDNA
antibodies

    The labeled cell extract was treated with
DNase before RNA-IP was performed because it is
possible that labeled DNAs, which are precipitated
by anti-dsDNA antibodies, are detected on the non-
denaturing polyacrylamide gel as background.
DNase-treated ^{32}P-labeled MOLT4 cell extract was
incubated with Protein A Sepharose 4B-bound
antibodies. Precipitated RNAs were analyzed on a
2.5% polyacrylamide non-denaturing gel: 33.H11 IgG
(1.0 μg); anti-dsDNA-positive patient sera LG, CC,
JP, IL, respectively; normal human serum DB; normal
human IgG from Con fraction II (1.0 μg); affinity-
purified anti-dsDNA IgG (1.0 μg each) from sera LG,
AP, CC, respectively; serum NK containing only
anti-Ro/La antibodies; serum FF containing only
anti-U1RNP/Sm antibodies; JC and MH sera containing
both anti-P and anti-dsDNA antibodies,
respectively; serum AR containing only anti-P
antibodies. 33.H11 (0.1 μg) was preincubated with
100 μg/ml each of Yeast tRNA, calf thymus dsDNA,
BSA, or the recombinant ribosomal protein S1 (G7-
RP). 33.H11 (0.1 μg) did not immunoprecipitate 18S
RNA when deproteinized MOLT4 cell extract was used
as the antigen. As shown in the Western blot, all
four sera containing anti-dsDNA antibodies
precipitated 18S RNA whereas normal human serum,
serum containing only anti-Ro/La, or only anti-
U1RNP/Sm antibodies did not. Moreover, 1.0 μg of
33.H11 and 1.0 μg each of affinity-purified anti-
dsDNA IgG precipitated 18S RNA while 1.0 μg of
normal IgG from Cohn fraction II did not. Two sera

33

containing both anti-P and anti-dsDNA antibodies
precipitated 18S RNA whereas the serum containing
only anti-P antibodies did not. Therefore, it was
concluded that anti-dsDNA antibodies

5   immunoprecipitate 18S ribosomal RNA specifically.

From the observation of RNA-IP using
deproteinized MOLT4 cell extract as the source of
antigen, the absence of 18S RNA precipitates by 0.1
μg of 33.H11 IgG indicates that proteins are

10   essential for 18S RNA immunoprecipitation by anti-
dsDNA antibodies. 1.0 μg of 33.H11 IgG did not
precipitate 18S RNA, either, when deproteinized
cell extract was used as the antigen.

A quantity (0.1 μg) of 33.H11, that reacts

15   strongly with both G7-RP and calf thymus dsDNA, was
preincubated with 100 μg/ml each of G7-RP, BSA,
calf thymus dsDNA, or Yeast tRNA before RNA-IP was
performed. As a result, 18S RNA
immunoprecipitation by 33.H11 was inhibited

20   completely by 100 μg/ml each of G7-RP and calf
thymus dsDNA whereas it was not blocked by the same
concentration of either BSA or tRNA. These results
suggest that G7-RP is essential for 18S ribosomal
RNA immunoprecipitation by anti-dsDNA antibodies.

Table II.   Inhibition of in vitro translation by anti-dsDNA Abs

Samples		CPM	(-) (%INHIB)	CPM	(+) (%INHIB)	Change
			DNase treatment of reticulocyte lysate			
no added IgG [mRNA(-)]		2,243	(___)	892	(___)	____
no added IgG [mRNA(+)]		60,777	(___)	46,966	(___)	____
<whole serum IgG>						
LG IgG (anti-dsDNA)	9μg	48,261	(20.6)	16,233	(65.4)	+44.8
AP IgG (anti-dsDNA)	9μg	56,796	(6.6)	23,358	(50.3)	+43.7
CC IgG (anti-dsDNA)	9μg	83,765	(0)	17,399	(63.1)	+63.1
FF IgG (anti-U1RNP/Sm)	9μg	72,558	(0)	47,477	(0)	0
NK IgG (anti-Ro/La)	9μg	79,896	(0)	48,678	(0)	0
DB IgG (normal)	9μg	91,999	(0)	45,598	(2.9)	+2.9
CW IgG (normal)	9μg	83,749	(0)	44,165	(6.0)	+6.0
<affinity-purified IgG>						
anti-dsDNA(LG) IgG	1μg	37,105	(38.9)	19,680	(58.1)	+19.2
	3μg	36,768	(39.5)	15,520	(67.0)	+27.5
anti-dsDNA(AP) IgG	1μg	34,539	(43.2)	12,864	(72.6)	+29.4
	3μg	35,555	(41.5)	12,659	(73.0)	+31.5
anti-dsDNA(CC) IgG	1μg	50,590	(16.8)	14,247	(69.7)	+52.9
	3μg	38,477	(36.7)	13,946	(70.3)	+33.6
33.H11 IgG	1μg	38,021	(37.4)	11,853	(74.8)	+37.4
	3μg	30,076	(50.5)	9,503	(79.8)	+29.3
Normal human IgG	1μg	74,470	(0)	48,714	(0)	0
(Cohn fraction II)	3μg	78,178	(0)	49,692	(0)	0

Each sample was tested after adding of 1, 3, or 9 μg of IgG.

CPM, average of duplicate translation runs (cpm in a 5-μl

reaction mixture).   %INHIB, %inhibition defined in the text.

Change, differences in % inhibition that resulted from the

incubation with DNase-treated lysate as compared to the

incubation with non-DNase-treated lysate.

Inhibition of *in vitro* translation by anti-
dsDNA antibodies

As shown in Table II, *in vitro* translation
of rabbit globin mRNA was not affected by the
addition of 9.0 µg of whole IgG from FF (anti-
UlRNP/Sm), NK (anti-Ro/La), DB (normal), CW
(normal) serum, or 1.0 and 3.0 µg or normal human
IgG (Cohn fraction II) when either DNase-treated or
non-treated reticulocyte lysate was used. The
small inhibition (2.9-6.0%) noted with these
negative control antibodies in the DNase-treated
reticulocyte lysate was considered to be
nonspecific inhibition. Nine µg of whole IgG from
anti-dsDNA-positive LG, AP, or CC serum, which
inhibited the translation poorly or not at all when
the lysate was not treated with DNase, inhibited
translation to a considerable degree (50-65%) when
the lysate was treated with DNase. However, only
1.0 µg of affinity-purified anti-dsDNA IgG [anti-
dsDNA(LG) and anti-dsDNA(AP)] or the monoclonal
anti-dsDNA 33.H11 IgG were required for translation
inhibition (38-43%) even if the lysate was not
treated with DNase. Moreover, this translation
inhibition by 1.0 µg of affinity-purified and anti-
dsDNA and 33.H11 IgG was uniformly and
significantly enhanced when the reticulocyte lysate
was treated with DNase (see Table II). As the
degrees of translation inhibition by 1.0 or 3.0 µg
of affinity-purified anti-dsDNA and 33.H11 IgG were
very similar, the inhibitory effect of anti-dsDNA
IgG was apparently maximal (67-80% when DNase-
treated lysate was used) with 3.0 µg of affinity-
purified anti-dsDNA IgG added. The quality of the
duplicate samples was reflected by the calculated
value of the Pearson correlation coefficient
r^2=0.972 for the whole data set.

Discussion

In the study of RNA-IP, it was demonstrated that anti-dsDNA antibodies precipitated only 18S RNA whereas normal human IgG and other autoantibodies (antibodies to UlRNP, Sm, Ro/SSA, La/SSB, or ribosomal protein P) did not. Also, this 18S RNA immunoprecipitation was inhibited by preincubating 33.H11 with calf thymus dsDNA or with the recombinant r-protein S1 (G7-Rp). Thus, it was concluded that anti-dsDNA antibodies precipitate 18S ribosomal RNA specifically. Moreover, it was demonstrated that r-protein S1 is essential for 18S RNA immunoprecipitation by anti-dsDNA antibodies. These results might support the evidence that r-protein S1 is located on the 40S ribosomal subunit (in eukaryotes) where 18S ribosomal RNA is an integral component. In Example 1, it was shown that the differences of the staining pattern by anti-dsDNA antibodies in IF (classical nuclear pattern versus nucleolar and cytoplasmic pattern represented by 33.H11) are due to the differences of anti-dsDNA Ab affinity between r-protein S1 and dsDNA. In the study described above, 33.H11, which has a higher affinity for r-protein S1 than for dsDNA, penetrate the plasma membrane, localizes in the cytoplasm, and remains there even after 48 hours of observation. From these standpoints, the observation of RNA-IP in this study suggests not only that the anti-dsDNA antibodies which have higher affinity for dsDNA can recognize r-protein S1 but also the possibility that anti-dsDNA antibodies recognize r-protein S1 in the cytoplasm after penetrating cell membranes. Furthermore, as RNA-IP is one of the methods for detecting antibodies to native RNA-related proteins, the results as described above indicate that anti-dsDNA

antibodies can recognize the native form of human
r-protein S1.

The r-protein S1 in *E.coli* is well known to
be associated with the 30S ribosomal subunit via
its N-terminal globular domain. It has been
reported that the major function of r-protein S1 of
E.coli in protein synthesis is at the mRNA binding
step, as described by Subramanian, *Prog. Nucleic
Acids Res*. Mol. Biol. 28:101 (1983). Thus this
protein plays a key role in translation initiation
of *E.coli*. The translation inhibition of globin
mRNA by anti-dsDNA antibodies was then determined.
There are to our knowledge no published studies
describing a role for r-protein S1 or its analogue
in eukaryotic cells but these experiments suggest
such a role. However, some anti-dsDNA antibodies
might have much higher affinity for dsDNA than for
r-protein S1. Therefore, the rabbit reticulocyte
lysate was treated with DNase to investigate more
sensitively the translation inhibition. The
conclusion from these data is that anti-dsDNA
antibodies inhibit the translation of globin mRNA
even when some dsDNA exist in the reticulocyte
lysate and that the inhibition is greatly enhanced
when free dsDNA is eliminated by DNase treatment.

It is possible that anti-dsDNA antibodies
inhibit the translation of mRNA by recognizing the
mRNA binding sites on the eukaryotic analogue of r-
protein S1 and by inhibiting the mRNA binding to r-
protein S1. Demonstration of a powerful inhibition
of protein synthesis by anti-dsDNA antibodies by
its interaction with the human r-protein S1
provides a mechanism for a pathogenic role of anti-
dsDNA antibodies after their penetration of living
cells.

**Exampl   3:   Demonstration of Broad Cross-
reactivity of an anti-Id Reagent with
Anti-dsDNA from SLE patients.**

Normal human serum was used as the source
of the anti-Id that was prepared by passage of
normal human serum over a column made with affinity
purified anti-dsDNA Fab from a single lupus
patient. Elution of the anti-Id with 3 M $MgCl_2$ and
pepsin digestion resulted in anti-Id Fab_2 which was
used to coat immulon plates. Five different
affinity purified polyclonal anti-dsDNA populations
were then shown to bind the anti-Id Fab_2 much more
strongly than to normal Cohn fraction FII Fab_2,
about two-fold on average. In addition two human
monoclonal IgG anti-dsDNA antibody samples bound
the anti-Id Fab_2 more strongly than control CFII
Fab_2. In all cases dsDNA, but not RNA, blocked the
interactions between the anti-Id Fab_2 and the
various anti-dsDNA preparations. Affinity purified
anti-Ro/SSA, anti-La/SSB, anti-U1RNP and normal
CFII IgG preparations bound anti-Id Fab_2 and control
CFII Fab_2 equally. This broad and specific cross-
reactivity of anti-dsDNA from different SLE
patients and an anti-Id (anti-anti-Id dsDNA) from a
normal person has not been previously recognized
and has potential as an immunologically specific
reagent to block and/or downregulate anti-dsDNA in
SLE.

Modifications and variations of the present
invention will be obvious to those skilled in the
art and are intended to come within the scope of
the appended claims.

We claim:

1.   A method for treating a lupus patient
characterized by the presence of anti-dsDNA
comprising administering to the patient a
therapeutic composition in a pharmaceutically
acceptable carrier for administration to a patient
selected from the group consisting of peptides
having sequence identity with ribosomal protein S1
which are immunoreactive with anti-dsDNA and anti-
idiotypic antibody or antibody fragments
immunoreactive with anti-dsDNA antibodies.

2.   The method of claim 1 wherein the
peptide is between four and forty amino acids in
length.

3.   The method of claim 1 wherein the
peptide is conjugated to a carrier molecule or is a
fusion protein.

4.   The method of claim 1 wherein the
composition is a peptide administered to a patient
in an amount effective to induce tolerance.

5.   The method of claim 1 wherein the
composition is anti-idiotypic antibody
immunoreactive with anti-dsDNA antibody.

6.   The method of claim 5 wherein the
antibody is administered in a dosage effective to
kill anti-dsDNA antibody producing cells.

7.   The method of claim 5 wherein the
antibody is administered in a dosage effective to
decrease the amount of anti-dsDNA antibody levels
in the patient.

8.   A therapeutic composition in a
pharmaceutically acceptable carrier for
administration to a patient selected from the group
consisting of peptides having sequence identity
with ribosomal protein S1 which are immunoreactive
with anti-dsDNA and anti-idiotypic antibody or
antibody fragments immunoreactive with anti-dsDNA antibodies.

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9. The composition of claim 8 wherein the peptide is between four and forty amino acids in length.

10. The composition of claim 8 wherein the peptide is conjugated to a carrier molecule or is a fusion protein.

11. The composition of claim 8 wherein the composition is a peptide administered to a patient in an amount effective to induce tolerance.

12. The composition of claim 8 wherein the composition is anti-idiotypic antibody immunoreactive with anti-dsDNA antibody.

13. The composition of claim 12 wherein the antibody is administered in a dosage effective to kill anti-dsDNA antibody producing cells.

14. The composition of claim 12 wherein the antibody is administered in a dosage effective to decrease the amount of anti-dsDNA antibody levels in the patient.
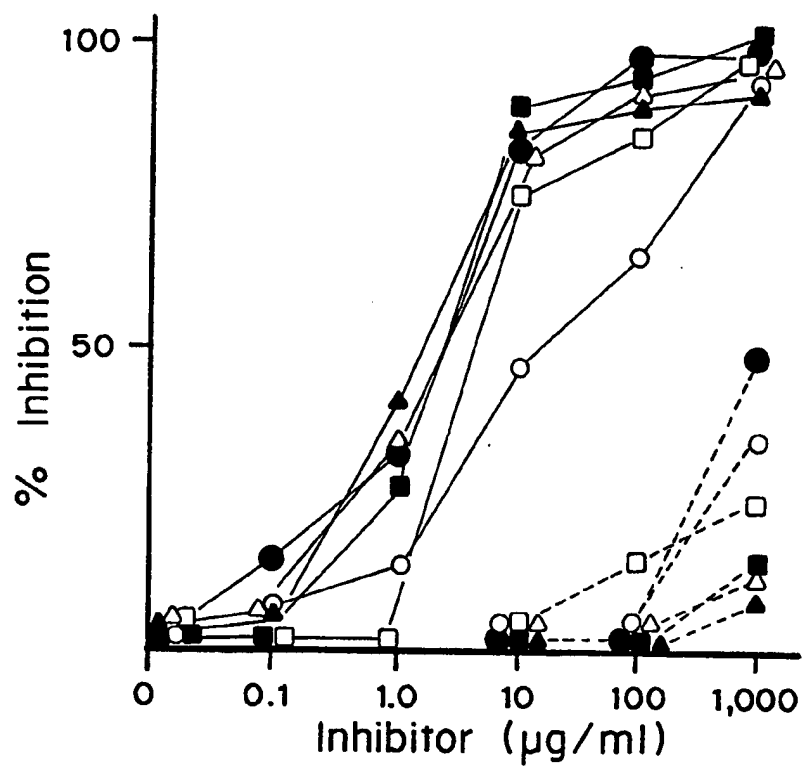
FIG. 1

FIG. 2

```
GGT GAA GAA GGA GTT GTG CCA GCA CGT GAG TAC TCA GAC GAT CGT  45
Gly Glu Glu Gly Val Val Pro Ala Arg Glu Tyr Ser Asp Asp Arg  [15]

AAC ATC AAC CTG GCA GAC GAA TTA AAA ATT GGT GAT ACC ATT GAA  90
Asn Ile Asn Leu Ala Asp Glu Leu Lys Ile Gly Asp Thr Ile Glu  [30]

GCA GTT GTC ATT TCT AAC GTA ACA AGC GAC AAG GAA GGC GTC AGT  135
Ala Val Val Ile Ser Asn Val Thr Ser Asp Lys Glu Gly Val Ser  [45]

TAC TTG TTG TCA AAG AAG CGT TTG GAT GCG CGC AAG GCA TGG GAA  180
Tyr Leu Leu Ser Lys Lys Arg Leu Asp Ala Arg Lys Ala Trp Glu  [60]

AAC TTG AGC TTT GCT GAA GGT GAC ACA GTT GAT GCC AAG GTT ATC  225
Asn Leu Ser Phe Ala Glu Gly Asp Thr Val Asp Ala Lys Val Ile  [75]

AAC GCT GTT CGT GGT GGT TTG ATT GTT GAT GTT AAC GGC GTA CGT  270
Asn Ala Val Arg Gly Gly Leu Ile Val Asp Val Asn Gly Val Arg  [90]

GGT TTC GTA CCA GCA TCA ATG GTT GCA GAA CGT TTC GTT TCT GAT  315
Gly Phe Val Pro Ala Ser Met Val Ala Glu Arg Phe Val Ser Asp  [105]

TTG AAC CAA TTC AAG AAT AAG GAT ATT AAA GCA CAA GTT ATC GAA  360
Leu Asn Gln Phe Lys Asn Lys Asp Ile Lys Ala Gln Val Ile Glu  [120]

ATT GAC CCT GCT AAT GCA CGT TTG ATT TTG TCA CGT AAG GCT GTT  405
Ile Asp Pro Ala Asn Ala Arg Leu Ile Leu Ser Arg Lys Ala Val  [135]

GCT GCA CAA GAA CGC GCT GCA CGA TTG GCT GAA GTA TTT AGC AAG  450
Ala Ala Gln Glu Arg Ala Ala Gln Leu Ala Glu Val Phe Ser Lys  [150]

TTG TCA GTT CGT GAA GTT GTT GAA GGA ACT GTT GCC CGT TTG ACA  495
Leu Ser Val Gly Glu Val Val Glu Gly Thr Val Ala Arg Leu Thr  [165]

GAC TTC GGC GCA TTC GTT GAC TTG GGT GGT GTT GAT GGT TTG GTT  540
Asp Phe Gly Ala Phe Val Asp Leu Gly Gly Val Asp Gly Leu Val  [180]

CAC GTA TCA GAA ATC TCA CAC GAT CGT GTG AAG AAC CCG GCC GAT  585
His Val Ser Glu Ile Ser His Asp Arg Val Lys Asn Pro Ala Asp  [195]

GTA TTG ACA AAG GGT GAC AAG GTT GAT GTT AAG ATC TTG GCA TTG  630
Val Leu Thr Lys Gly Asp Lys Val Asp Val Lys Ile Leu Ala Leu  [210]

GAC ACT GAA AAG GGT CGT ATC TCA TTG TCA ATC AAA GCA ACA CAA  675
Asp Thr Glu Lys Gly Arg Ile Ser Leu Ser Ile Lys Ala Thr Gln  [225]
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FIG. 2a

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CGT GGA CCT TGG GAC GAA GCT GCA GAT CAA ATC GCT GCA GGT TCA 720
Arg Gly Pro Trp Asp Glu Ala Ala Asp Gln Ile Ala Ala Gly Ser [240]

GTG CTT GAA GGT ACT GTT AAG CGT GTG AAG GAC TTT GGT GCC TTT 765
Val Leu Glu Gly Thr Val Lys Arg Val Lys Asp Phe Gly Ala Phe [255]

GTT GAA ATT TTG CCT GGT ATC GAA GGT CTT GTG CAC GTG TCA CAA 810
Val Glu Ile Leu Pro Gly Ile Glu Gly Leu Val His Val Ser Gln [270]

ATT TCA AAC AAG CGT ATT GAA AAC CCA TCA GAA GTT TTG AAG TCT 855
Ile Ser Asn Lys Arg Ile Glu Asn Pro Ser Glu Val Leu Lys Ser [285]

GGT GAC AAG GTA CAA GTG AAG GTA TTG GAC ATT AAG CCA GCC GAA 900
Gly Asp Lys Val Gln Val Lys Val Leu Asp Ile Lys Pro Ala Glu [300]

GAA CGT ATT TCA TTG TCA ATG AAG GCT TTG GAA GAA AAG CCA GAA 945
Glu Arg Ile Ser Leu Ser Met Lys Ala Leu Glu Glu Lys Pro Glu [315]

CGT GAA GAT CGT CGT GGT AAC GAT GGT TCA GCT TCA CGT GCT GAT 990
Arg Glu Asp Arg Arg Gly Asn Asp Gly Ser Ala Ser Arg Ala Asp [330]

ATC GCT GCT TAC AAG CAA CAA GAT GAC TCA GCC GCA ACA TTG GGT 1035
Ile Ala Ala Tyr Lys Gln Gln Asp Asp Ser Ala Ala Thr Leu Gly [345]

GAC ATC TTT GGT GAT AAG TTG TAA GAGGCATCAACATAAAAGAGCTGGTTC 1086
Asp Ile Phe Gly Asp Lys Leu ***                                [352]

GCCAGTTCTTTTATTTTTGAAGAAAAATTGAGTGGGCATTAGTGGGCGCTCACGGTATG   1145

AAAAAGGAGGTGCGATTATGGCAGCACCAGTAGTAGCCATTGTTGGCGACCAAACGTCG   1204

GAAAATCGACTATCTTTAACCGGATGGCCGGAGAACGTATTGCAATTGTTGAAGATCAA   1263

CCAGGGGTAACACGCGATCGTTTGTACGCGCCAGCCGAATGGTTGAATTAT          1314
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FIG. 3

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HS1  63  SFAEGDTVDAKVINAVRGGLIVDVNGVRGFVPASMVAERFVSDLNQFKNKDIKAQVIEI 121
ES1 101  AYEDAE--TGVINGK-K--FT-ELD-I-A-L-G-L-DV-P-R-TLHLEG-ELEFK--KL 159
RS1 107  K-EA-ER-EGI IF-Q-K--FT--LD-AVA-L-R-Q-DI-PIR-VTPADAQPAALRNLKM 165
PS1   1        E--TGVINGK-K--FT-EL-I-A-L-G-L-DV-P-R-TTHLEG-ELEFK--KL  54
CS1 182      --DVV-KG-IVG-NK--VVAL-E-L-----F-QISSK-SAEELLE-E-PLKFV--V 236
                          a            b                   c
                         ****        ******                ***

HS1 122  DPANARLILSRKAVAAQERAAQLAEVFSKLSVGEWEGTVARLTDFGAFVDLGGVDGLV 180
ES1 160  -QKRNNVVV-R--IES-NS-ERDQLLEN-QE-ME-K-I-KN--Y-----------L 218
RS1 166  -KRRGNIVV--RT-LEES--E-RS-IVQN-EE-Q-----V-KNI--Y-----I--L 224
PS1  55  -QKRNNVVV-R--IES-SS-ERDQLLEN-QE-ME-K-I-KN--Y-----------L 113
CS1 237  -EEQS--VM-NRKAM-DSQ-M-DSQAQ-GI-S--T----QS-KPY---I--I--IN--L 287
              d                                e
             ****                           ******

HS1 181  HVSEISHDRVKNPADVLTKGDKVDVKILALDTEKGRISLSIKATQRGPWDEAADQIAAG 239
ES1 219  -ITDMAWK---H-SEIVNV--EIT--V-KF-R-RT-V--GL-QLGED--VAI-KRYPE- 277
RS1 225  --TDMAWR---H-SEIQNI-QQ-K-Q-IRINQ-TH----GM-QLESD--GIGAKYPV- 283
PS1 114  -ITDMAWK---H-SEIVNV--EIT--V-KF-R-RT-V--GL-QLGED--VAI-KRYPE- 172
CS1 288  ---Q-----SDI-T--QP--TLK-M-SH-R-R--V---T-KLEPT-G-            337
              a      b             c               d
             ****  ******        ******           ****
```

FIG. 3a

```
HS1   240 SVLEGTVKRVKDFGAFVEILPGIEGLVHVSQISNKRIENPSEVLKSGDKVQVKVLDIKP   298
ES1   278 TK-T-R- TNLT -Y- C ----EE -V- - - - - -EM RD-V-DATL--SV -- E- EA- FTGVDR   510
RS1   284 KKIS - - - TNIT -Y- - - - -LE- - - - -I - I -EM -RPG-QVI-EFNK --V- RAV- - - -VDV   430
PS1   173 TK-T-R - TNLT -Y- C ----EE -V- - - - - -EM                  -K-- E IAAV - - QVDA   319
                                    e
                                  *****

HS1   299 AEERISLSMKALEEKPERE 317
ES1   511 KNRA- - - - -VR-KD-AD-KD 529
RS1   431 DK - - - - -GI-QL       442
PS1   320 ER - - - - -GV-QLA-DP     335
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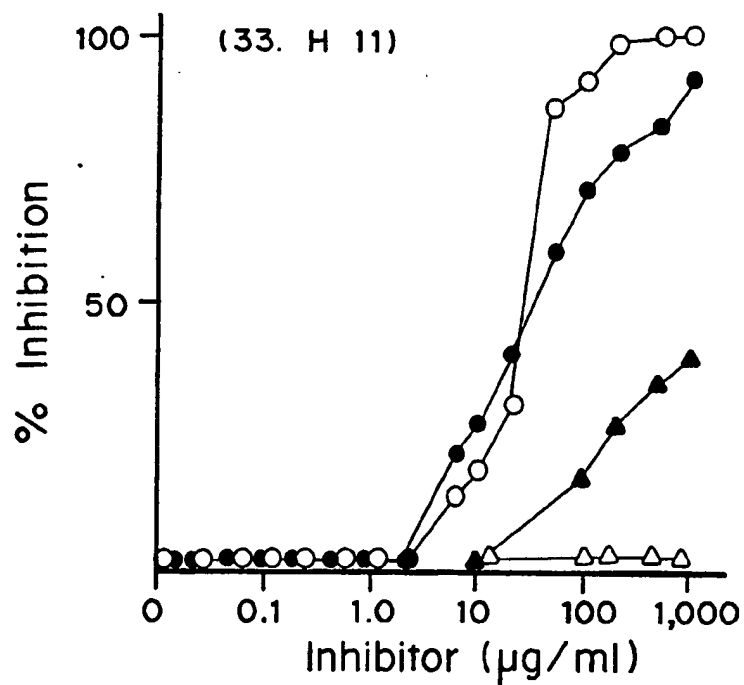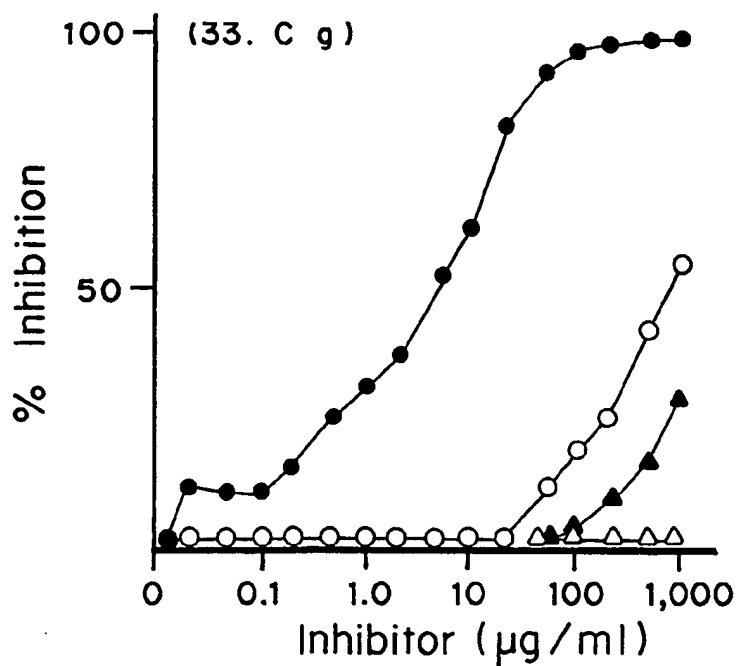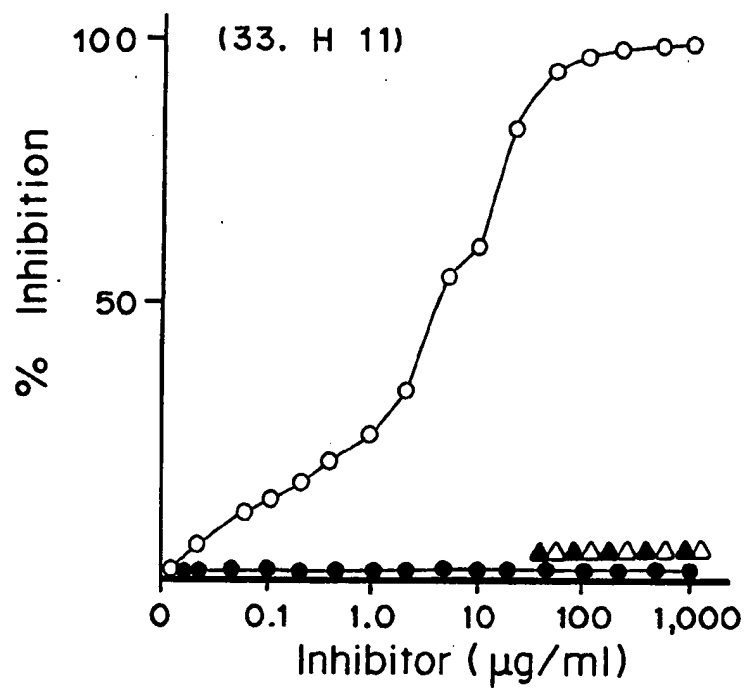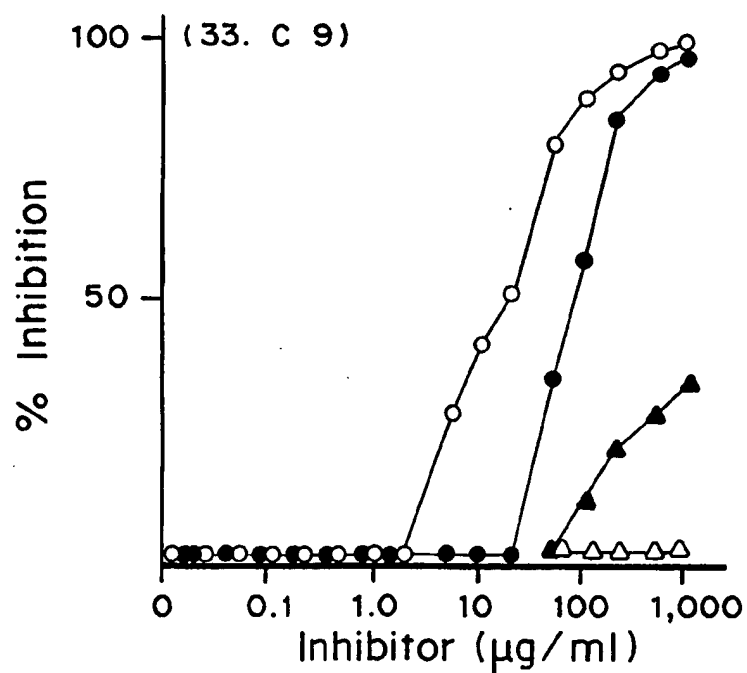
FIG. 4a

FIG. 4b

FIG. 4c

FIG. 4d

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61K39/395   A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SPRINGER SEMINARS IN IMMUNOPATHOLOGY, vol. 7, no. 1, 1984, BERLIN, GERMANY, pages 25-34, XP000605499 B. HAHN: "Suppression of autoimmune diseases with anti-idiotypic antibodies: Murine lupus nephritis as a model." see page 32, line 14 - line 23	1,5-8, 12-14
X	IMMUNOLOGY, vol. 74, no. 2, October 1991, OXFORD, GB, pages 342-347, XP000608109 C. RAVIRAJAN ET AL.: "Involvement in lupus disease of idiotypes Id.F-423 and Id.IV-228 defined, respectively, upon foetal and adult MRL/Mp-1pr/1pr DNA-binding monoclonal autoantibodies." see page 346, left-hand column, line 1 - line 6	1,5-8, 12-14

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

18 October 1996

Date of mailing of the international search report

0 5. 11. 96

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Nooij, F

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INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 83, no. 1, January 1991, OXFORD, GB, pages 126-132, XP000608102 C. MORLAND ET AL.: "Anti-idiotype and immunosuppressant treatment of murine lupus." see abstract	1,5-8, 12-14
X	THE JOURNAL OF IMMUNOLOGY, vol. 142, no. 4, 15 February 1989, BALTIMORE, MD, USA, pages 1159-1165, XP002016364 T. SASAKI ET AL.: "In vitro manipulation of human anti-DNA antibody production by anti-idiotypic antibodies conjugated with neocarzinostatin." see abstract	1,5-8, 12-14
A	NUCLEIC ACIDS RESEARCH, vol. 19, no. 15, 11 August 1987, OXFORD, GB, page 4297 XP002016365 D. GRABOWSKI ET AL.: "Drosophila AP3, a presumptive DNA repair protein, is homologous to human ribosomal associated protein P0." see the whole document	1-4,8-11
A	WO 94 21669 A (MEDCLONE, INC.) 29 September 1994 see the whole document	1-4,8-11
A	EP 0 113 431 A (YESHIVA UNIVERSITY) 18 July 1984 see claims	1,5-8, 12-14
P,X	ARTHRITIS & RHEUMATISM, vol. 38, no. 9 suppl., September 1995, NEW YORK, NY, USA, page S274 XP000608103 K. TSUZUKA ET AL.: "Lupus autoantibodies to double-stranded DNA cross-react with ribosomal protein S1." see abstract 726	1-4,8-11
P,X	THE JOURNAL OF IMMUNOLOGY, vol. 156, no. 4, 15 February 1996, BALTIMORE, MD, USA, pages 1668-1675, XP002016366 K. TSUZUKA ET AL.: "Lupus autoantibodies to double-stranded DNA cross-react with ribosomal protein S1." see abstract	1-4,8-11

INTERNATIONAL SEARCH REPORT

In   ational application No.

PCT/US 96/ 07597

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claim(s) 1 - 7
   is(are) directed to a method of treatment of the human/animal
   body, the search has been carried out and based on the alleged
   effects of the compound/composition.

2. ☐ Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter   nal Application No

PCT/US 96/07597

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		AU-B-	566246	15-10-87
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